User Manual

miniPERM®

SARSTEDT

Contents

Introduction 26
Design and function of the miniPERM® bioreactor

Operating instructions 28
Example of hybridoma cell culture

High density cell culture in the miniPERM® bioreactor 34
High density cell culture
Oxygen requirement of hybridoma cells
Production rate
Changing the medium
Handling of the miniPERM® bioreactor

Troubleshooting 35
Problems and solutions

Literature 39
Publications and application notes

Accessories 40

Ordering information 41

Brief instruction manual 42

1. Introduction

Design of the miniPERM® bioreactor

miniPERM® is a bioreactor made of two connected modules designed for the cell culture of hybridoma and other cells in high densities (“high density culture”). It consists of:

- a disposable **production module** which serves as a culture chamber with a volume of 35 ml or 50 ml (miniPERM® classic or HDC 50); and
- an autoclavable, reusable or sterile disposable **nutrient module** made of plastic (polycarbonate) which serves as a media reservoir with 550 ml volume that is attached to the production module.

To separate the two modules, the production module is fitted with a semi-permeable **dialysis membrane** (MWCO 12.5 kDa) on the side facing the nutrient module, when the two modules are connected.

The outer part of the production module is made of a thin, gas-permeable **silicone membrane**.

The screw cap of the nutrient module has an integrated **gas-exchange membrane**.

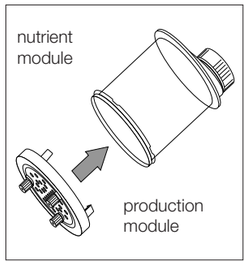
Function of the miniPERM® bioreactor

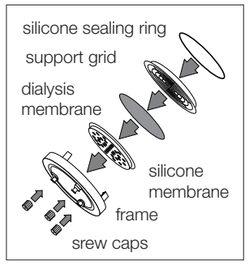
1. Dialysis membrane

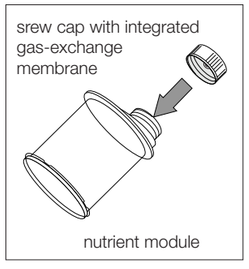
Neither the cells nor high molecular weight products released by the cells, e.g. secreted monoclonal antibodies, can pass through the semi-permeable dialysis membrane.
However, nutrients (glucose, amino acids), vitamins, ions, and gases (O_2, CO_2) dissolved in the medium can diffuse from the nutrient module into the production module. Due to more than a 10 fold excess of nutrient medium, the cells are provided with substances necessary for cultivation over a long period of time.

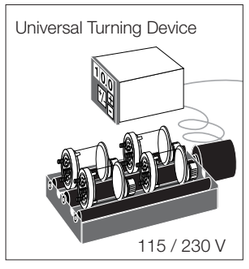
Simultaneously, acidic (e.g. lactic acid), toxic (e.g. ammonium ions) and other low molecular mass products of the cell metabolism diffuse from the production module through the dialysis membrane into the nutrient module where they are diluted and neutralised in the large excess of medium.

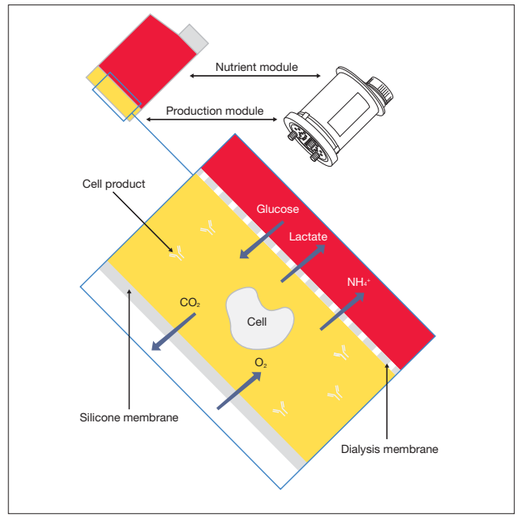
The discharge of CO_2 from the miniPERM® is further promoted by the fact that CO_2 (dissolved in the culture medium both physically and in the form of $NaHCO_3$) can pass through the dialysis membrane from the production module to the nutrient module.











The dialysis membrane is covered with a support grid facing the nutrient module. This grid has two functions:

It protects the dialysis membrane from mechanical damage, and provides a means of swirling the nutrient medium at the dialysis membrane surface leading to an improved diffusion of nutrients and metabolic products.

User Manual - miniPERM®

2. Silicone membrane

Oxygen requirements are fulfilled by O_2 diffusing from the incubator atmosphere into the production module through the silicone membrane. The CO_2 produced by the cells in corresponding quantities leaves the production module via the same route. The high CO_2 permeability of the silicone membrane in the production module means the $NaHCO_3$ in the medium is at equilibrium with the CO_2 in the incubator atmosphere.

3. Screw cap with gas-exchange membrane

The gas-exchange membrane in the screw cap of the nutrient module enables equilibration of pressure between the bioreactor and the outer environment.

4. Universal Turning Device

For optimal supply of nutrients and removal of metabolic waste products through the membranes, cells and the medium must be circulated continuously. Therefore, the miniPERM® bioreactor is rolled in a Universal Turning Device during cultivation.

Due to the exceptionally high cell densities produced in the miniPERM®, a higher than usual turning speed is required. For this reason, we recommended the use of the Universal Turning Device which can rotate the miniPERM® from 0.1 to 40 rpm compared to standard turners that usually have a maximum speed of 2.5 rpm.

Operating Instruction

All the operations described below must be carried out in a sterile hood!

Cells are cultivated under normal laboratory conditions, centrifuged and adjusted to the specified density.

Ensure the clips of the production module are completely snapped!

It is very important that the nutrient module is autoclaved to a maximum of 121°C and no more than 10 times. Higher temperatures lead to deformation of the polycarbonate.

Before connecting the two modules, unscrew the screw cap of the nutrient module (to prevent pressure build-up)!

Ensure the clips of the production module are completely snapped!

1. Preparation of cells for culturing in the miniPERM® bioreactor using hybridoma cells as example

The miniPERM® classic culture can be started with about 35 to 37 ml of a cell suspension containing approximately 1 to 5 x 10^5 cells/ml (35 to 185 x 10^6 cells total) (fig. 1). In most cases, the contents of 1 to 2 well grown 150 cm^2 cell culture flasks are sufficient for this purpose.

For using the miniPERM® HDC 50 production module the cell suspension contains 1 to 5 x 10^6 cells/ml in 50 ml.

2. Preparation of the miniPERM® bioreactor

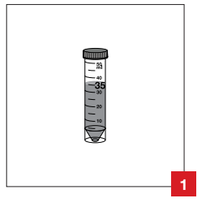
2.1 Pre-assembled miniPERM® sterile

In this version, the miniPERM® has been assembled from the two modules and sterilized. This represents a single-use product. It is not autoclavable. It can be used without further treatment (fig. 2).

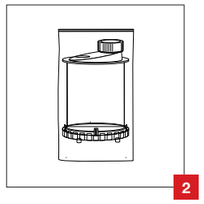
2.2 Multiple-use miniPERM®

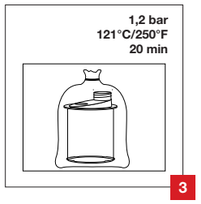
The production modules are supplied as disposables in an alu pouch. The reusable nutrient module must be sterilized by autoclaving to a maximum of 121°C prior to use.

- Pack the nutrient module in an autoclaving bag (fig.3) and autoclave it without the screw cap for 20 min to a maximum of 121°C (also refer to item 7).
- The alu pouch of the production module is opened to ensure the production module is resting on the screw caps of the Luer Lock-connectors. The production module should be left in the plastic pack!
- The autoclaved nutrient module is now pushed against the sealing ring of the production module (figs. 4 and 4a) until the snapper clips of the production module are snapped audibly into the indentations on the rim of the nutrient module.
- The plastic pack of the production module can now be removed.
- The assembled miniPERM® is now ready for use.

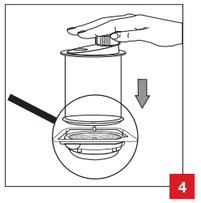


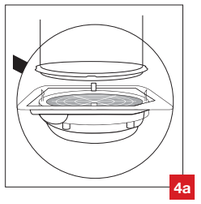
1


2



3


4


4a

When the cell suspension is introduced the air displaced by the medium must be released through one of the other ports! Therefore, a second screw cap should be loosened.

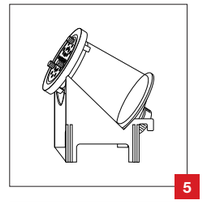
Medium at the rim of the filling port must be thoroughly removed (by drawing up with an exhauster before closing the port).

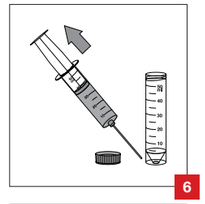
3. Filling the miniPERM® bioreactor

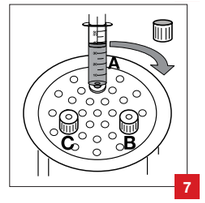
3.1 Introducing the cell suspension into the production module

The cell suspension is introduced into the production module with a syringe through one of the three ports equipped with Luer Lock connectors:

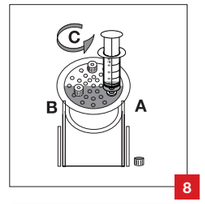
- Mount the miniPERM® bioreactor on the stand with the production module on top so that one of the three Luer Lock connectors is positioned at the highest point (fig. 5).
- Draw up the prepared cell suspension (5, 35 or 50 ml) through a sterile filling tube (5") using a disposable syringe (≤ 50 ml). Remove air bubbles from the syringe before injecting the cell suspension (fig. 6).
- Remove the screw cap at the uppermost point (A). Screw the syringe (without the filling tube) onto the Luer Lock connector (fig. 7).
- Turn the miniPERM® bioreactor so that one of the other screw caps is positioned at the highest point.
- Loosen a second screw cap at the uppermost point (C). Inject the cell suspension slowly into the production module (fig. 8).
- Close the Luer Lock connector of the port at position C tightly with a screw cap (fig. 9).
- Turn the miniPERM® bioreactor back so that the port (A) to which the syringe has been attached is positioned at the uppermost point again (fig. 10).
- Remove the syringe. Air bubbles should be removed by pressing the silicone membrane.
- Close the production module by tightly screwing a screw cap or a septum cap (see Troubleshooting) onto the Luer Lock connector of the third port in position A (fig. 11).


5

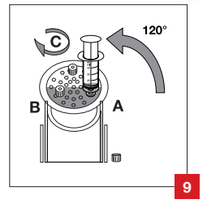

6



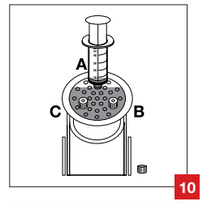
7



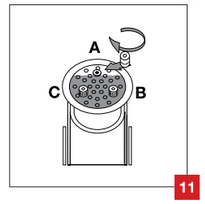
8



9



10



11

Do not fill more than 400 ml into the nutrient module. An air space must be maintained above the nutrient medium for successful cultivation.

The rolling speed must be selected according to the robustness of the cells, e.g.

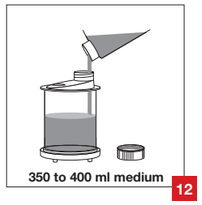
- murine hybridoma cells: 5 to 20 rpm
- human hybridoma and transfected cells: 2 to 10 rpm

Since the diameter of the miniPERM® bioreactor is twice the size of the rolls of the Universal Turning Device, miniPERM® rotates at half the set speed.

3.2 Introducing the medium into the nutrient module

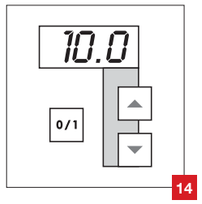
The nutrient module is filled with medium through the large neck opening which is fitted with a screw cap as follows:

- Remove the screw cap from the neck opening of the nutrient module and pour in 350 to 400 ml of tempered nutrient medium (fig. 12).
- To remove pressure in the miniPERM® bioreactor, press your hand against the silicone membrane of the production module (fig. 13).
- Close the neck opening with the screw cap. Tighten the screw cap applying a slight pressure only.
- Place the miniPERM® bioreactor onto the Universal Turning Device and set the speed at the Control Unit on 0.1 to 40 rpm.



12

13



14

The above rolling speeds refer to the display on the Control Unit. miniPERM® actually rotates at half this speed.

Under no circumstances should the screw caps of the three Luer Lock connectors of the production module be opened when the silicone membrane is extended! The excess pressure in the nutrient module would cause the cell suspension to spurt out and be lost. In addition, the outside of the miniPERM® bioreactor would be contaminated.

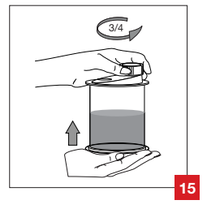
Should foam accumulate in the production module which is often the case when FCS is used as a medium supplement, it is difficult to loosen the screw cap of the production module without getting foam into the thread. To solve this problem, an antiFOAM® agent can be used in the nutrient module (see Troubleshooting).

Medium at the rim of the harvest port must be thoroughly removed (by drawing up with an exhauster) before closing the port.

4. Taking samples

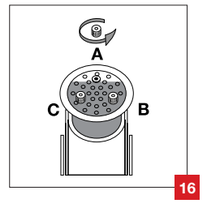
4.1 Taking samples through a port of the production module

- Place the miniPERM® bioreactor in a sterile hood.
- Ensure that pressure is released. Hold the miniPERM® bioreactor with one hand applying light pressure to the silicone membrane. With the other hand loosen (do not unscrew) the screw cap of the nutrient module, so excess pressure is released (see figs.15/15a). Close tightly again.
- Position the miniPERM® bioreactor on the stand so that one of the ports of the production module is at the highest point (A) and remove the screw cap of this connector (fig. 16).
- Fit a syringe of suitable size (approx. 1 ml, depending on the sample volume to be taken) onto the Luer Lock connector in position A (fig. 17).
- Turn the miniPERM® bioreactor so that the sampling port (A) with the attached syringe is positioned below the level of the liquid in the production module (fig. 17). Draw up the sample into the syringe.
- Before removing the syringe, miniPERM® bioreactor must be turned so that the sampling port with the attached syringe is positioned at the uppermost point (fig. 18).
- After removing the syringe air bubbles should be removed by pressing the silicone membrane with the fingers.
- Close the Luer Lock connector of the sampling port with a sterile screw cap (fig. 19) and return the miniPERM® bioreactor to the incubator.

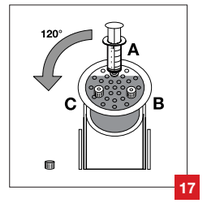


15

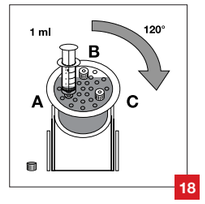
15a



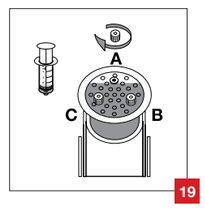
16



17



18



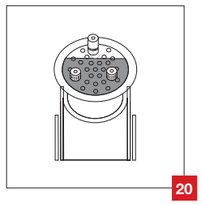
19

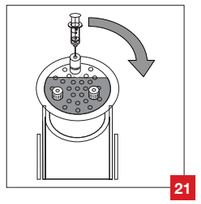
Small diameter needles (25G x $^5/_8$") should be used in order to retain the sterile barrier. The septum caps should be replaced after they have been pierced 5 or 6 times.

4.2 Taking samples through a septum

One of the three ports can be replaced with a septum cap which maintains the sterile barrier for sample removal.

- Prepare the miniPERM® bioreactor as described in 4.1.
- Clean the hole of the septum cap with a pad soaked in 70% pharmaceutical ethanol.
- Pierce a sterile needle with syringe (≤ 5 ml) in the hole of the septum cap.
- Turn the miniPERM® bioreactor so that the septum cap with the attached syringe is positioned below the level of the liquid in the production module (fig. 21).
- Draw the sample into the syringe.
- Remove the needle from the septum cap.
- Clean the hole of the septum cap with a sterile pad soaked in 70% pharmaceutical ethanol.


20


21

5. Harvesting

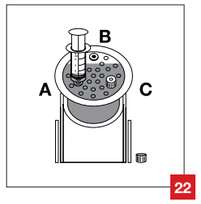
Harvesting must be quick to prevent the cells from settling and clumping.

Medium at the rim of the harvest port must be thoroughly removed (by drawing up with an exhauster)!

The interval and volume of harvesting depend on the cell density and the amount of product which the cells produce. Once or twice a week on an average, $^2/_3$ of supernatant is harvested. Harvesting is done essentially the same way as sampling.

- Prepare the miniPERM® bioreactor as described in 4.1.
- In order to prevent a negative pressure in the production module whilst drawing up the harvest with a syringe, it is necessary to loosen a second screw cap at position B (fig. 22).
- Draw the harvest into syringe.
- Close the second port (B) with a sterile screw cap.
- Turn the bioreactor so that the harvesting port (A) with the attached syringe is positioned at the uppermost point and remove the syringe.
- The volume extracted should be replaced by fresh medium, see 3.1.

Depending on the cell density reached the harvested cells could be resuspended in fresh media and reinjected into the production module.



22

Cleanliness is of utmost importance when changing the medium.

Risk of contamination! Care must be taken to ensure that no traces of the medium have been left on the thread of the filling port or run down the sides of the nutrient module. Should this occur, remove via an exhauster or with a sterile pad soaked with 70% pharmaceutical ethanol. Do not flame.

For disinfectants of the screw cap do not use ethanol, propanol or disinfectants/solvents that contain alcohol as their use will impair function of the PTFE membrane. Rinse with distilled water only.

The reusable screw cap can be autoclaved for 20 min to a maximum of 121°C at least 5 times without change to the material.

For disinfection use only 70% pharmaceutical grade ethanol or isopropanol for the miniPERM® bioreactor, the Universal Turning Device, the incubator and water bath.

All other chemicals, especially quaternary ammonium complexes may cause microcracks.

Do not use n-propanol!

6. Changing the medium in the nutrient module

Replacing the spent medium with fresh medium follows the same procedure as described in 3.2 for the first filling of the nutrient module.

- Place the miniPERM® in a sterile hood.
- Unscrew the screw cap of the neck opening on the nutrient module.
- Pour the used medium into a disinfected waste bottle (fig. 23).
- Fill 350 to 400 ml of fresh tempered (37°C) medium into the nutrient module (fig. 24).
- Remove pressure in the miniPERM® following the same procedure as described in 3.2 for pressure equilibration.
- Close the neck opening with a sterile screw cap. Tighten the screw cap applying a slight pressure only.

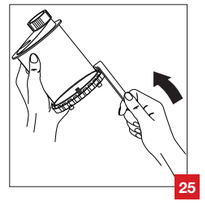
23

24

7. Cleaning and sterilisation of the nutrient module (multiple-use nutrient module)

The reusable nutrient module can be autoclaved at least ten times without changes to the material. To avoid damage to the polycarbonate, the nutrient module should be placed freely, i.e. not subjected to pressure in the autoclave. The maximum autoclave temperature is 121°C.

- The modules are separated at the end of the culture, using the hook end of the opener provided, to gently prise open the snapper clips. The production module can then be easily removed from the nutrient module (fig. 25).
- Clean the nutrient module only with hot water and neutral or weak acid or alkaline detergent without rinsing agents. Rinse with ample water to completely remove the detergent. Do not use an automatic dish washer.
- Autoclave the nutrient module without the screw cap for 20 min, to a maximum of 121°C. Do not use any alkaline-based corrosion protector in autoclave boiler water.


25

8. Cleaning the Universal Turning Device

The Universal Turning Device can be disinfected with a cloth soaked in 70% pharmaceutical grade ethanol or isopropanol. Do not use n-propanol. Do not autoclave the Universal Turning Device.

High density cell culture in miniPERM®

'High density cell culture'
The design of the miniPERM® bioreactor makes it possible to culture cells to considerably higher densities ('high density cell culture') than in conventional stationary culture in which the oxygenation of the cells can only be achieved via gas exchange through the cap. Consequently, the cellular products are secreted in much higher concentrations compared to conventional cultures.

Cells cultured in high density are very much dependent on optimal culture, so they are more sensitive to disturbances than cells in conventional stationary culture at densities of 10^5 to 10^6 cells/ml.

Due to the high density (10^7 cells/ml and greater) the cells are dependent on the continuous supply of large quantities of nutrients and oxygen and on the removal of metabolic waste products and CO_2.

Oxygen requirement of hybridoma cells
The oxygen consumption rate of hybridoma cells is in the order of 5µg per 10^6 cells per hour.

With a cell density of 10^7 cells/ml, (which is easily attainable in the miniPERM® bioreactor), the oxygen requirement of the 35 x 10^7 cells cultivated in the 35 ml (miniPERM® classic) of the production module is approximately 1.75 mg/hour.

Production rate
To produce a certain amount of product (monoclonal antibodies in the case of hybridoma cells), a certain number of cells are needed. The number of cells required depends on the individual properties of the cells cultured. According to Fazekas de St. Groth (J. Immunol. Methods 57, 1983, 121) hybridoma cells typically produce between 4 x 10^7 and 7 x 10^8 antibody molecules per cell in a 24-hour period.

Changing medium
The stressful conditions of high density cell culture means the cells require a medium of particularly high quality with respect to the content of glucose and $NaHCO_3$. The content of glucose should not be less than 4 g/l. If less, the medium should be supplemented with additional glucose. Most media have an $NaHCO_3$ content (DMEM 3.78 g/l) which ensures a buffer capacity up to 2 weeks. Due to the optimal gas exchange in the miniPERM® bioreactor and the high cell density the initial glucose content would be exhausted before the pH colour change of the nutrient medium is indicated from red to yellow. Thus, the buffer capacity of the medium to neutralize and to buffer metabolic waste products is exhausted.

The interval of the medium change depends on the cell line, the medium and the increase of the cell density. We suggest 1 to 2 times per week. An orange-yellow colour of the medium in the production module is indicative of a high cell density (> 10^7 cells/ml).

Handling of the miniPERM® bioreactor
The temperature of the nutrient medium should always be brought to 37°C before the module is filled. After miniPERM® is filled with nutrient medium and introduced to the incubator, the air above the medium heats and expands. This expansion can be substantial: per 1°C in temperature the increase in volume is 1/273 of the volume at 0°C. Thus, the volume increases by about 24 ml if the temperature in the space above the medium in the nutrient module rises from 4°C to 37°C. This increase in volume would cause a substantial rise in pressure (more than 0.1 bar) in the nutrient module of the miniPERM®. This would lead to expansion of the silicone membrane of the production module.

There is another effect typical for cultures with $NaHCO_3$-buffered media in a closed bioreactor which causes a further rise of pressure in the nutrient module. When a culture is started, there is usually atmospheric air rather than the CO_2 containing incubator atmosphere in the air space above the medium of the nutrient module. Therefore, the $NaHCO_3$ in the medium decomposes, and the CO_2 is released into the space above the medium of the nutrient module. The result is a further rise in pressure in the nutrient module and alkalinisation of the medium.

These differences in overall and CO_2 partial pressure will be levelled off by diffusion of gases through the gas-exchange membrane of the screw cap for the nutrient module. Consequently, the pH value will be readjusted to physiological levels within a few hours.

Troubleshooting

Problem	Solution
The cells are not growing (after a few days, the percentage of dead cells is above 70%)	• Increase the initial cell density. • Produce the starter cell suspension for the miniPERM® culture with $^1/_3$ ml conditioned medium from a cell culture flask and $^2/_3$ ml fresh medium. • Increase the percentage of the serum supplement in the medium. • Decrease the rolling speed when using sensitive cells, e.g. of human hybridoma cell lines. Set the rolling speed to just 0.5 rpm. • Use of cellPROTECT® (Ordering Information see page 41). cellPROTECT® protects the cells against shear forces. (Add 0.05 to 0.1% to the medium in the production module.) • Do not fill more than 400 ml medium into the nutrient module. An air space must be maintained above the nutrient medium in the bioreactor.
Culturing of adherent cells	• In the miniPERM® classic, some adherent cells (e.g. CHO) grow in the suspension in an aggregate form. This has no influence on the productivity of the cells. • Use of the miniPERM® SM production module. This module has 2 integrated stationary matrices made of polyester. • Use of Microcarrier beads for the production of secreted recombinant proteins. The cells grow on the microcarrier in the miniPERM® classic production module.
The cells do not reach high densities (< 1 x 10^7 cells/ml)	• Cells of some lines do not grow to high densities (less than 2.0 x 10^7 cells/ml). If it is possible to cultivate the cells at average cell densities and high viability over a long time period by regular changes of the medium in the nutrient module, high antibody concentration can be obtained.
Cells aggregate and die	• The cells must always be kept in suspension. If the miniPERM® bioreactor is removed from the Universal Turning Device and placed in the sterile hood, the cells will immediately begin to settle. In this case, they may form clusters and possibly die. To avoid such an occurrence, all necessary manipulations must be carried out as quickly as possible.
Accumulation of foam: If too much foaming occurs in the nutrient and production modules, it is not possible to release the pressure without getting foam on the screw caps.	• Use an antifoam agent, e.g. antiFOAM®, Ordering Information see page 41. (Add 0.5 to 1 ml to the nutrient module.) • Reduce the serum concentration in the medium contained in the nutrient module and, if possible, also in the production module.

Problem	Solution
Duration of the miniPERM® culture	• This depends on the growth kinetics of the cell clone. By regularly changing the medium and regularly harvesting the cells, the miniPERM® bioreactor culture can be operated in a continuous way over several weeks. The timing of the medium exchange and cell harvesting will vary from cell line to cell line.
Leakage: 1. Between production and nutrient module During assembly of the miniPERM®, the snapper clips on the production module are snapped audibly into the indentations on the rim of the nutrient module. Do not attempt to twist the two modules together.	• After assembly, ensure that all the snapper clips are fully snapped with their corresponding indentations on the rim of the nutrient module. A second check will ensure that nutrient and production modules are located correctly. • The reusable nutrient module should be autoclaved to a maximum temperature of 121°C. It should be used no more than 10 times, otherwise this leads to deformation and cracking of the polycarbonate. • To avoid damage to the polycarbonate, the nutrient module should be placed into the autoclave in a way as to be contact-free from any other item. • To sterilize the miniPERM® bioreactor, use only 70% pharmaceutical grade ethanol or isopropanol. **DO NOT USE** any other chemicals; especially quaternary ammonium complexes may cause microcracks. • Tighten the screw cap of the neck opening of the nutrient module applying slight pressure only. • Autoclave the reusable screw cap separately to a maximum of 121°C and no more than 5 times without change of the material. The sterile single use screw caps are not autoclavable.
2. At the screw cap of the neck opening of the nutrient module	• Do not flame the neck opening of the nutrient module and the screw caps. This will lead to deformation of the polycarbonate. • Each time the nutrient module is opened, reclose with a new screw cap.
Pressure: The silicone membrane of the production module is always expanded outwards, due to a build-up of pressure in the bioreactor.	• The humidity inside the CO_2 incubator may be too low. Make sure that the humidity is at least 90%. • The temperature of the nutrient medium should always be brought to 37°C before the module is filled. If the nutrient module has been filled with cold medium (4°C) and the miniPERM® bioreactor has been placed into the incubator, the air above the medium heats up and expands. This will lead to expansion of the silicone membrane of the production module. • Equilibration of pressure. For this, loosen the nutrient module screw cap by ¾ turn and afterwards tighten applying slight pressure only.
The silicone membrane of the production module is pulled inward.	• This may be due to a bacterial or fungal contamination in the nutrient or production modules.

Problem	Solution
Contamination: 1. In the production module	First check the components (cell suspension, medium, culture vessels, incubator etc.) for sterility. Cleanliness is of utmost importance when taking samples, harvesting cells or changing the medium. • Use a septum port to take samples. Replace one screw cap on the production module with a septum (Ordering Information see page 41). • Following sampling or cell harvesting, the access ports must be free of culture medium before closing with the screw caps. • Always use a new screw cap for each sampling/harvest port which has been opened.
2. In the nutrient module	• Use of an antifoam agent, (e.g. antifoam®, Ordering Information see page 41). If foam has accumulated in the production module, (which is often the case when FCS is used as a medium supplement), it is difficult to loosen the screw caps of the production or nutrient modules without getting foam into the threads. This could be a potential risk of contamination. • After exchanging the culture medium, the neck opening must be free from any such medium prior to closing with a screw cap. • Always use a new screw cap for the nutrient module after changing the medium. • If contamination is limited to the nutrient module, the culture can be saved. Remove the cells from the production module and transfer them to another sterile miniPERM® bioreactor.
Rotation speed	The turning speed must be selected according to the robustness of the cells. • Generally, the bioreactor has to be rotated at low speed at the beginning of the culture. With an increase of cell density the rolling speed can be increased concomitantly. Examples: • murine hybridoma cells: 5 to 20 rpm (Display 10-40) • human hybridoma and transfected cells: 2 to 10 rpm (Display 4-20) The rolling speeds refer to the display on the Control unit. miniPERM® actually rotates at half this speed.
The rollers of the Universal Turning Device stop working	• Ensure that the Universal Turning Device is correctly assembled. • After cleaning the turning device with pharmaceutical grade ethanol or isopropanol you must oil the bearings. Do not use n-propanol. Caution! Keep the rubber material of the rollers free from oil in order to prevent the bioreactors from slipping from the device. • Do not autoclave the Universal Turning Device.

Problem	Solution
Medium Changing the medium	• Under conditions of stress, in each high density cell culture the cells require a medium of particular high quality with regard to the content of glucose and $NaHCO_3$. In the miniPERM® bioreactor, the cells can be cultivated with the same $NaHCO_3$-dependent media normally used in tissue culture. The content of glucose should be not less than 4 g/l, otherwise the medium should be additionally supplemented accordingly. • For the production of proteins, the medium should be changed in intervals of up to 7 days. For the production of biomass, the medium should be changed in intervals of up to 5 days. • The medium in the nutrient module should be replaced as soon as there is a slight change in colour from salmon-pink to a yellowish-pink. This signifies that the buffering capacity of the medium is reaching the point of exhaustion.
Serum	• Under high density cell culture conditions, serum concentration can be critical. In the miniPERM® bioreactor the serum concentration should be not less than that used in a stationary culture of the same cell line. • For the production of monoclonal antibodies or other proteins, a serum concentration between 5 to 30% should be used. For the production of biomass, a serum concentration of 10% is generally sufficient. • In the miniPERM® nutrient module the serum concentration can be halved, and in some cases it can be reduced to zero. • It is also possible to cultivate hybridoma or other cell types in the miniPERM® bioreactor. The cells must be adapted to a serum or protein free environment.

Publications & Application Notes

Bruce, M.P; Boyd, V.; Duch, C; White, J.R.
Dialysis-based bioreactor systems for the production of monoclonal antibodies -alternatives to ascites production in mice.
Journal of Immunological Methods 264, No. 1-2, (06/2002) 59-68

Caulfield, J.J.; Fernandez, M.H.; Sousa, A.R.; Lane, S.J.; Lee, T.H.;Hawrylowicz, C.M.
Regulation of major histocompatibility complex class II antigens on human alveolar macrophages by granulocyte-macrophage colony-stimulating factor in the presence of glucocorticoids
Immunology 98 (1999) 104-110

Falkenberg, F.W.
Production of monoclonal antibodies in the miniPERM® bioreactor: comparison with other hybridoma culture methods
Res. Immun. 6/149 (1998) 560-570

Falkenberg, F.W.; Weichert, H.; Krane, M.; Bartels, I.; Palme, M.; Nagels, H.-O.; Fiebig, H.
In vitro production of monoclonal antibodies in high concentration in a new and easy to handle modular minifermenter
J. Immun. Meth. 179 (1995) 13-29

Kagan, E.; Vieira, E.; Petrie, H.T.
Comparison of hollow fiber bioreactors and modular minifermentors for the production of antobodies
CAAT-OPRR Workshop on Alternatives in Monoclonal Antibody Production, 9/1997, Haborplace Hotel Baltimore, MD

Little, M.; Kipriyanov, S.M.; Le Gall, F.; Moldenhauer, G.
Of mice and men: hybridoma and recombinant antibodies
Review Immunology Today (08/00) 364-370

Metzger, J.; Nicklisch, N.; Schmidt, B.; Kufer, P.; Peschel, C.; Bernhard, H.
Induction of a T helper cell response against the tumor associated antigen HER-2 using monocyte-derived dendritic cells
ESACT-Meeting 09/2001, Sweden

Raulf-Heimsoth, M.; Sander, I.; Zhiping, Ch.; Borowitzki, G.; Diewald, K.; van Kampen, V., Baur, X.
Development of a monoclonal antibody-based sandwich ELISA for detection of the latex allergen Hev b Int.
Arch. Allergy Immunol. 123 (2000) 236-241, 8/2000

Schütt, C.; Fürll, B.; Stelter, F.; Jack, R.S.; Witt, S.
CHO transfectants produce large amounts of recombinant protein in suspension culture
Journal of Immunological Methods 204 (1997) 99-102

Vollmers, H.P.; Dämmrich, J.; Wozniak, E.; Bier, D.; Müller-Hermelink, H.K.
Apoptotic in vitro and in vivo activity of the human monoclonal antibody SC-1 on stomach cancer cells
2nd International Gastric Cancer Congress, Munich, Germany, 04/1997
Konstantinov, M; Mindova, M.; Gospodinov, P.; Genova, P.
Three-Dimensional Bioreactor Cultures: A Useful Dynamic Model for the Study of Cellular Interactions
N.Y. Acad. Sci. 1030: 103-115 (2004)

Dewar, V.; Voet, P.; Denamur, F.; Smal, J.
Industrial Implementation of in Vitro Production of Monoclonal Antibodies
ILAR Journal, Volume 46, Number 3 (2005)

Berlin, V.; Rousselle, P.
Production of a recombinantly expressed laminin fragment by HEK293-EBNA cells cultured in suspension in a dialysis-Based Bioreactor
Protein Expr. Purif 48(1): 43-48, 2006

Application notes

Vollmers, H.P.; Wozniak, E.
Cultivation of human hybridoma cell line in the miniPERM® bioreactor
University of Würzburg, Germany

Tutas, M.
Cultivation of mouse hybridoma cells in the miniPERM® bioreactor
Cell Diagnostica GmbH, Germany

Lingnau, A.
Cultivation of IgM producing hybridoma cells in the miniPERM® bioreactor
VM-PRO GmbH, Luckenwalde, Germany

Schliephacke, T.; Käppel, S.
Long-term proliferation of CHO cells in the miniPERM® bioreactor
iOnGen AG, In vitro Systems Services GmbH, Germany

Müller, S.
HEK-U293 cells cultivated in suspension in the miniPERM® bioreactor
Knoll AG Ludwigshafen, Germany

Prestle, J.; Ott-Gebauer, S.
Production of recombinant adenoviruses in the miniPERM® bioreactor
University of Göttingen, Germany

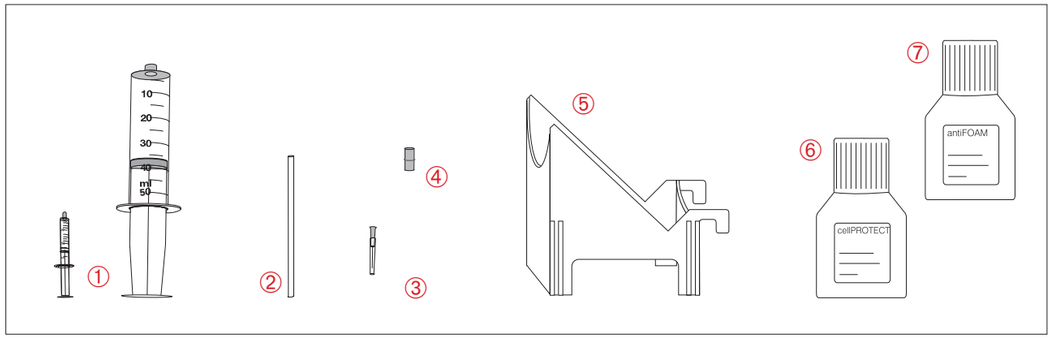
Sponaas, A.; Anding, P.
Cultivation of a murine macrophage like cell line (K774) in the miniPERM® bioreactor
Max-Planck-Institut for Infections Biologie, Berlin, Germany

Dobner, T.
Cultivation of insect cells (SF9 cells - baculovirus system) in the miniPERM® bioreactor
University of Regensburg, Germany

Schillo, M.; Meyer, N.; Bentrop, J.
Cultivation of insect cells (Drosophila Schneider cells [S2]) in the miniPERM® bioreactor
University of Karlsruhe, Germany

Wuppermann, F.
Cultivation of a murine hybridoma cell line in miniPERM® classic and miniPERM® HDC 50 bioreactors
LCTech GmbH, Dorfen, Germany

miniPERM® Accessories



The following accessories are available for convenient handling during cultivation:

① Sterile single-use 50 ml Luer Lock and 2 ml Luer syringes

For simple and safe cell suspension inoculation, sampling and harvesting.

② Sterile filling tube 5"

The sterile filling tube offers a simple solution to cell suspension transfer (e.g. from centrifuge tubes to single-use syringe). A filling tube is slightly flexible, connects directly to a 50 ml Luer syringe and has a sufficiently large inner diameter to minimize shear-stress induced cell damage.

③ Luer syringe needles 25G x 5/8"

For sampling and supplementing the medium in the production module via septum ports.

④ Septum ports

To simplify sampling and supplementing procedures and to further reduce contamination risks, a replacement of one or two of the three standard screw caps on the production module with sterile septum ports is possible.

The rubber septum ports offer the possibility for addition of fluids to or removal of samples from the production module using sterile single-use syringe needles of small diameter. There is no need to open the module. The septum ports should be replaced after they have been pierced 5 or 6 times.

⑤ miniPERM® stand

For mounting the miniPERM® bioreactor during inoculation, sampling and harvest.

⑥ cellPROTECT®

cellPROTECT® increases the viscosity of the culture medium and thereby protects the cells against shear-stress. cellPROTECT® is added to the cell suspension in the production module at concentrations of 0.05 to 0.1% v/v.

⑦ antiFOAM®

When serum-containing medium is used, significant quantities of foam may develop in both modules of the miniPERM®. By adding 0.5 to 1 ml antiFOAM® to the nutrient module medium, this foam build-up is reduced.

Start-up Support Kit

Accessories to inoculate, sample and harvest.
Consists of:

- Sterile single-use 50 ml Luer Lock syringe (8x)
- Sterile single-use 2 ml Luer syringe (20x)
- Sterile filling tube 5”, Luer (8x)
- Luer syringe needles (25G x 5/8"), sterile (20x)
- Septum ports, sterile (6x)
- miniPERM® stand (1x)
- cellPROTECT®, 1 ml, sterile (1x)
- antiFOAM®, 5 ml, sterile (1x)

Ordering Information

	miniPERM® classic Vol. 35 ml		miniPERM® HDC 50 Vol. 50 ml		miniPERM® SM 240 cm²	
	Order No.	Packaging	Order No.	Packaging	Order No.	Packaging
Bioreactor, sterile	94.6001.059	12	94.6077.121	12	94.6077.618	12
Test Kit*, sterile	94.6077.009	1	94.6077.122	1	94.6077.609	1
Production Module, sterile	94.6001.055	12	94.6077.017	12	94.6077.616	12
Nutrient Module	94.6001.153	4				

*4 Bioreactors + **Start-up Support Kit

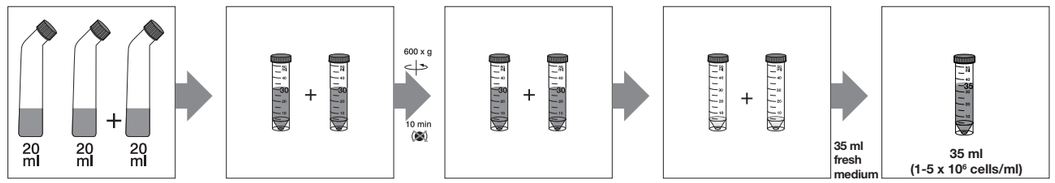
Order No.	miniPERM® Accessories		Packaging (bag/box)
94.6001.153	Nutrient Module for miniPERM®, autoclavable		4
94.6001.054	Stand for miniPERM®		4
94.6001.036	Screw Cap for Production Module, sterile		6
94.6077.037	Screw Cap for Nutrient Module, sterile		16
74.4312	Septum Ports, sterile		100
94.6077.135	Luer Syringe Needles 25 G x 5/8” (0.5 x 16 mm), sterile		100
94.6077.136	Single use 2 ml Luer Syringe, sterile		100
94.6077.137	Single use 50 ml Luer Lock Syringe, sterile		60
94.6077.138	Filling Tube 5”, Luer, sterile		100
94.6077.320	antiFOAM®, sterile, 100 ml		1
94.6077.041	cellPROTECT®, sterile, 100 ml		1
94.6001.094	Start-up support kit**	*Pieces*	1
	• Single use 50 ml Luer Lock Syringe, sterile	*8*	
	• Single use 2 ml Luer Syringe, sterile	*20*	
	• Filling Tube, sterile	*8*	
	• Luer Syringe Needles 25 G x 5/8”, sterile	*20*	
	• Septum Ports, sterile	*6*	
	• Stand for miniPERM®	*1*	
	• cellPROTECT®, 1 ml, sterile	*1*	
	• antiFOAM®, 5 ml, sterile	*1*	

Order No.	Universal Turning Device	Packaging
94.6001.061	Universal Turning Device 115 / 230 V	1

Brief instructions for use

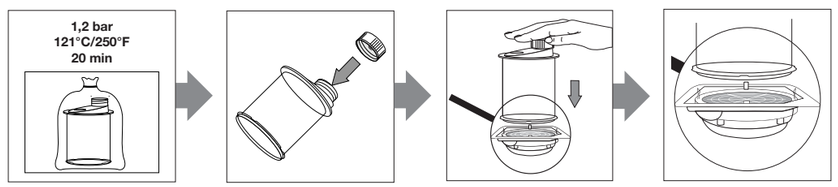
For example: Preparation of cells for cultivation in miniPERM® classic production module with 35 ml volume.
For using the miniPERM® HDC 50 production module 1-5 x 10^6 cells/ml in 50 ml medium are prepared.

Preparation of cells

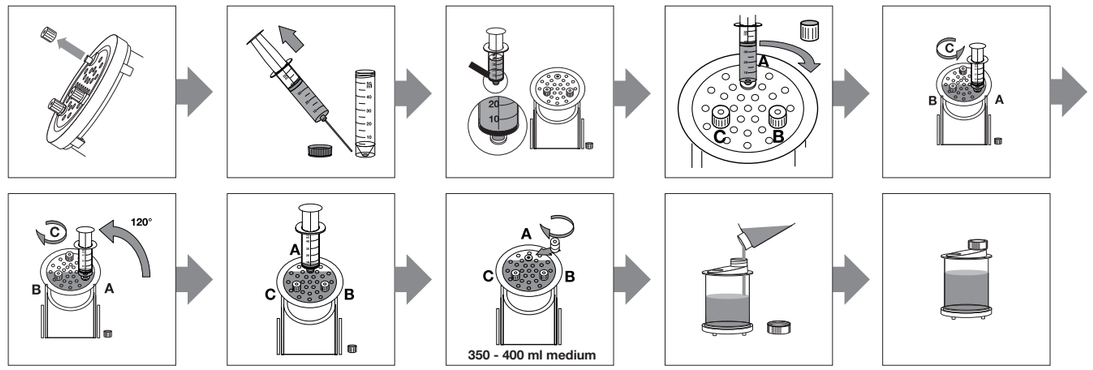


miniPERM® preparation

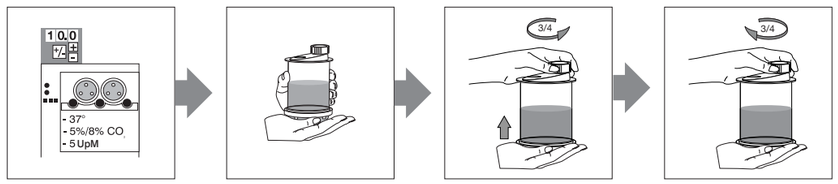
(The sterile bioreactor option is ready for use without further preparation).
Autoclavable nutrient module:



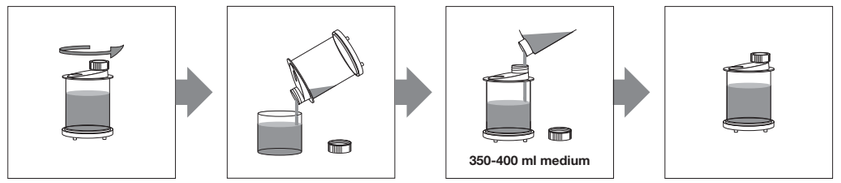
Filling



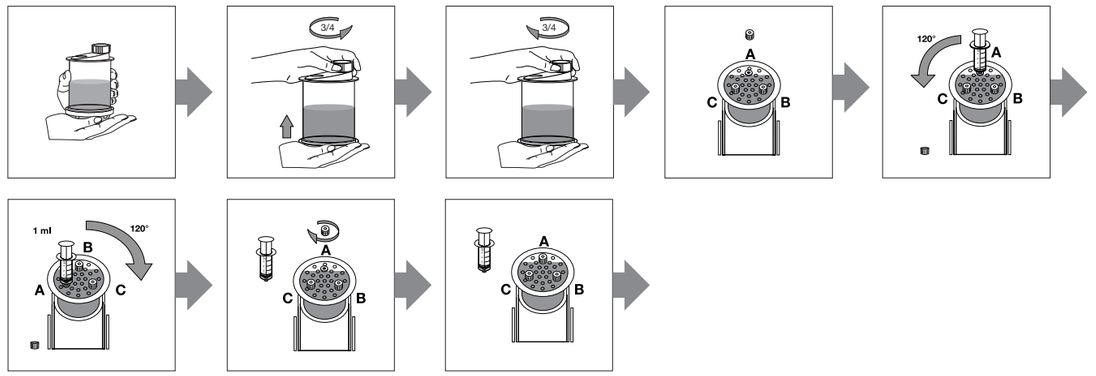
Conditioning



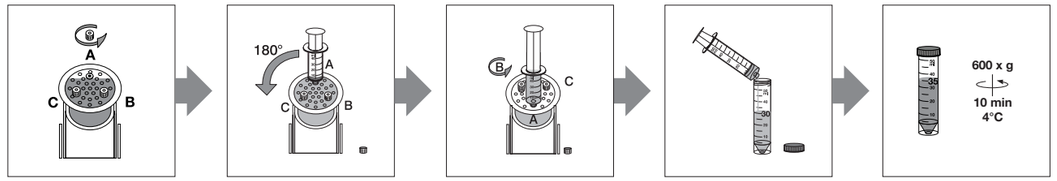
Medium change



Sampling



Harvesting



SARSTEDT AG & Co.
P.O. Box 12 20
D-51582 Nümbrecht
Phone +49 2293 3050
Fax +49 2293 305-122
info@sarstedt.com
www.sarstedt.com

SARSTEDT

Technical modifications reserved

GB 135-0413-0413